

# Protecting Groups for Nucleosides Used in Synthesizing Oligonucleotides<sup>1</sup>

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**Abstract:** Three protecting groups useful in syntheses involving nucleosides are described. These are  $\beta$ -benzoylpropionyl and benzoylformyl for OH groups of nucleosides and isobutyloxycarbonyl for NH<sub>2</sub> of the cytosine ring. The preparation and properties of 3'-O- and 5'-O-( $\beta$ -benzoylpropionyl)thymidine, 3'-O-benzoylformylthymidine, and N<sup>6</sup>-isobutyloxycarbonyl-3'-O-( $\beta$ -benzoylpropionyl)deoxycytidine are described.

The  $\beta$ -cyanoethyl phosphotriester route for the stepwise synthesis of oligonucleotides utilizes a condensation of a 3'-O- $\beta$ -cyanoethylphosphorylnucleoside (or oligonucleotide derivative) with a nucleoside.<sup>2</sup> Since the activated phosphoryl can attack the 3'-OH as well as the 5'-OH of the nucleoside, a protecting group for the 3'-OH must be used if substances with 3'-5' internucleotide links are to be formed exclusively in this step. The 3'-O protecting group must be sufficiently stable to survive in aqueous pyridine and in pyridine solutions containing arenesulfonyl chlorides and phosphorylating reagents; yet it must be susceptible to cleavage under conditions that do not disturb a methoxytrityl ether, which is sensitive to acid, or a  $\beta$ -cyanoethyl phosphoric ester, which is sensitive to alkali.

None of the available blocking groups met the requirements. A search for new blocking groups was directed toward substituents which could be selectively labilized by interaction with a specific reagent in solution. Of the various possibilities,  $\beta$ -benzoylpropionyl appeared most attractive. The keto function in esters of  $\beta$ -benzoylpropionic acid should serve as a site for a facile reaction with hydrazine, and the nucleophilic NH<sub>2</sub> group in the resulting derivative should be positioned favorably to attack and cleave the ester function. In accord with these considerations, Curtius reported many years ago that equimolar amounts of ethyl  $\beta$ -benzoylpropionate and hydrazine hydrate react exothermically to yield 4,5-dihydro-6-phenylpyridazine.<sup>3</sup>

We found that esters derived from  $\beta$ -benzoylpropionic acid and primary or secondary alcohols are cleaved quantitatively within a few hours by dilute solutions of hydrazine hydrate (0.5 M) in pyridine and acetic acid (4:1 v/v) at room temperature. This solution behaves as an essentially neutral medium. It is neither sufficiently acidic to attack *p*-monomethoxytrityl ethers nor sufficiently basic to attack  $\beta$ -cyanoethyl phosphoric esters. Moreover, hydrazine in 4:1 pyridine-acetic acid does not affect the purine and pyrimidine rings of the nucleosides under the conditions used to remove the  $\beta$ -benzoylpropionyl group.<sup>4</sup>

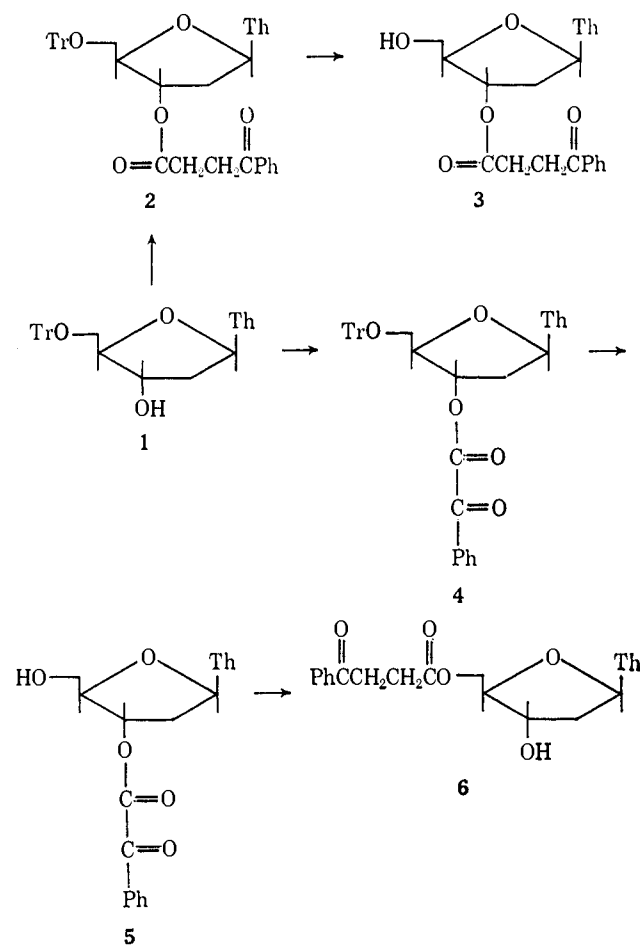
(1) Part XIV in series on nucleotide chemistry. For a preliminary account see R. L. Letsinger, M. H. Caruthers, P. S. Miller, and K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 7146 (1967). The research was supported by the Division of General Medical Sciences, National Institutes of Health (GM 10265) and by a predoctoral fellowship awarded to P. S. Miller (SF1-GM34033).

(2) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **91**, 3350 (1969).

(3) T. Curtius, *J. Prakt. Chem.*, [2], **50**, 529 (1895).

(4) R. L. Letsinger, P. S. Miller, and G. W. Grams, *Tetrahedron Letters*, 2621 (1968). Hydrazine hydrate in aqueous solution attacks

To test the utility of  $\beta$ -benzoylpropionyl as a blocking group, we prepared 3'-O-( $\beta$ -benzoylpropionyl)thymidine (3) and 5'-O-( $\beta$ -benzoylpropionyl)thymidine (6). The former was obtained by reaction of 5'-O-tritylthymidine or 5'-O-(*p*-monomethoxytrityl)thymidine



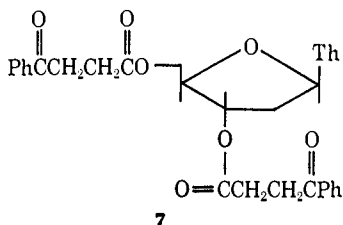
with excess  $\beta$ -benzoylpropionic acid and dicyclohexylcarbodiimide in pyridine followed by hydrolytic removal of the 5'-O protecting group. For preparation of the latter, 5'-O-tritylthymidine was treated with benzoylformyl chloride. The resulting derivative (4) was hydrolyzed with aqueous acetic acid to give 3'-O-benzoylformylthymidine (5), which, in turn was esterified with  $\beta$ -benzoylpropionic acid. The benzoylformyl group was then selectively removed by hydrolysis with

the cytosine and uracil rings: P. A. Levene and M. L. Bass, *J. Biol. Chem.*, **71**, 167 (1926); F. Baron and D. M. Brown, *J. Chem. Soc.*, 2855 (1955); V. Habermann, *Biochem. Biophys. Acta*, **55**, 999 (1962).

aqueous pyridine at room temperature. This synthesis depends upon the fact that  $\beta$ -benzoylpropionic esters are stable in aqueous pyridine whereas benzoylformic esters are attacked by aqueous pyridine.

Both **3** and **6** yielded thymidine and 4,5-dihydro-6-phenylpyridazine when treated with hydrazine hydrate in the pyridine-acetic acid mixture. Benzoyl and acetyl derivatives of thymidine are stable under these conditions. That  $\beta$ -benzoylpropionyl will effectively protect an oxygen function was demonstrated by small-scale reactions of **3** and **6** with excess  $\beta$ -cyanoethyl phosphate and dicyclohexylcarbodiimide in pyridine. On alkaline hydrolysis and assay by paper chromatography thymidine 5'-phosphate ( $R_f$  0.83 in solvent D) was found as the phosphorylation product from **3** and thymidine 3'-phosphate ( $R_f$  0.96 in solvent D) was obtained as the phosphorylation product from **6**. No diphosphate derivatives were found. Similarly, acetylation of **3** and **6** with acetic anhydride in pyridine followed by treatment with hydrazine in pyridine-acetic acid yielded 5'-O-acetylthymidine ( $R_f$  0.25; tlc, EtOAc) and 3'-O-acetylthymidine ( $R_f$  0.33; tlc, EtOAc), respectively.

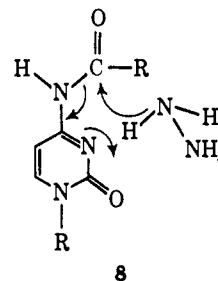
Reaction of thymidine with excess  $\beta$ -benzoylpropionic acid and dicyclohexylcarbodiimide afforded a mixture of 3'-O,5'-O-bis( $\beta$ -benzoylpropionyl)thymidine (**7**) and 5'-O-( $\beta$ -benzoylpropionyl)thymidine. Cleavage of the diester with a limited amount of hydrazine was not selective; both **3** and **6** were recovered from the products.



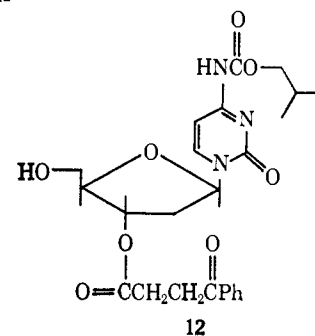
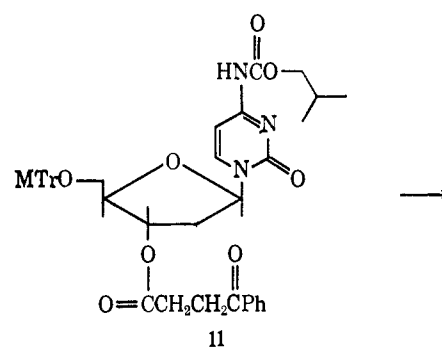
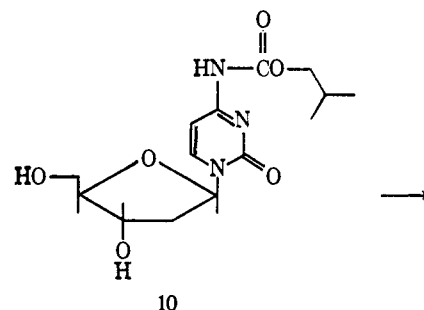
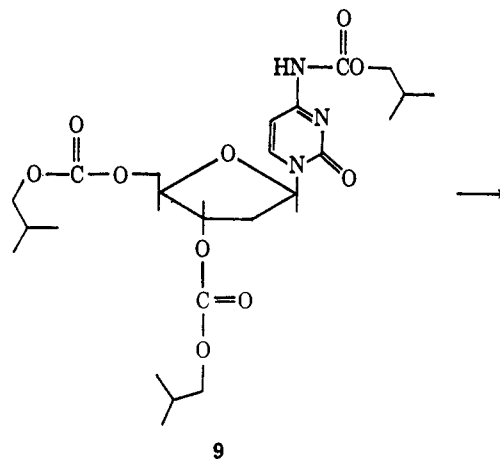
When work with the  $\beta$ -benzoylpropionyl group was extended to the other common deoxyribonucleosides, a complication was encountered: it was found that hydrazine in pyridine-acetic acid not only cleaves benzoylpropionic esters but also removes benzoyl groups from the N<sup>6</sup> position of deoxycytidine and deoxyadenosine.<sup>4</sup> Since the amino groups in these nucleosides must be protected during phosphorylation reactions, this fact required that some group other than benzoyl be used at the N<sup>6</sup> position in the course of an extended oligonucleotide synthesis.

The unusual reactivity exhibited by hydrazine toward N-benzoyl derivatives of deoxycytidine and deoxyadenosine may be attributed to two factors: (1) a low-energy pathway is available for transferring a proton from the attacking nucleophile to the departing group (see formula **8**),<sup>4</sup> and (2) hydrazine is a highly reactive nucleophile.<sup>5</sup> On the assumption that cleavage of the C-N bond would be more difficult in carbamate derivatives, we investigated the properties of N-isobutyloxycarbonyl derivatives of deoxycytidine. Cleavage of the carbonyl-oxygen bond in these derivatives would not be expected under mild conditions since no low energy pathway for transfer of a proton from hydrazine to the isobutoxy group is available.

(5) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 1778 (1960).



The reaction of deoxycytidine with excess isobutyl chloroformate yielded the trisisobutyloxycarbonyl derivative (**9**), which on brief treatment with sodium hydroxide in dioxane-water was converted to N<sup>6</sup>-isobutyl-



oxycarbonyldeoxycytidine (10). This compound indeed proved to be resistant to hydrazine. It was recovered unchanged after standing in 0.5 M hydrazine hydrate in 4:1 pyridine-acetic acid for a period of 48 hr.

To be useful in synthesis, of course, the protecting group must be susceptible to cleavage. Fortunately, in spite of the low reactivity toward hydrazine, N<sup>6</sup>-isobutyloxycarbonyldeoxycytidine was found to react with concentrated ammonium hydroxide. Under the conditions used to remove N-benzoyl groups (concentrated ammonium hydroxide for 2 days)<sup>6</sup> compound 10 was converted completely to deoxycytidine. Care was taken to establish the identity of the product since ammonia might have displaced the isobutoxy group to form a urea derivative. The compound was characterized by a mixture melting point with deoxycytidine and by the infrared spectrum as well as by chromatographic data. In addition, the presence of the amino group was confirmed by a reaction with ninhydrin.<sup>7</sup>

For use in synthesizing oligonucleotides, N<sup>6</sup>-isobutyloxycarbonyl-3'-O-( $\beta$ -benzoylpropionyl)deoxycytidine (12) was prepared from 10. This was accomplished by converting 10 to the corresponding 5'-O-(*p,p'*-dimethoxytrityl) derivative, esterifying this compound with  $\beta$ -benzoylpropionic acid, and hydrolyzing the 5' ether with aqueous acetic acid. The over-all yield of 12 from 5.26 g of deoxycytidine hydrochloride was 2.96 g (30%) for a sequence in which the intermediates were carried through without purification at each step.

Experiments with deoxyadenosine indicate that isobutyloxycarbonyl is also a satisfactory protecting group for NH<sub>2</sub> in this nucleoside. These experiments will be described in a subsequent paper.

## Experimental Section

The equipment and chromatographic techniques were described in the preceding paper.<sup>3</sup> Analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and by Miss Hilda Beck, Northwestern University. Solvents used in paper chromatography were: A, isopropyl alcohol-ammonium hydroxide-water (7:1:2); D, 2 M hydrochloric acid-*n*-propyl alcohol (1:3). Thin layer chromatography (tlc) was carried out on Eastman chromagram sheets 6060 with ethyl acetate (EtOAc) or ether as the solvent.

**3'-O-( $\beta$ -Benzoylpropionyl)-5'-O-tritylthymidine (2).** A mixture of 0.800 g (1.65 mmol) of 5'-O-tritylthymidine,<sup>8</sup> 0.885 g (4.95 mmol) of  $\beta$ -benzoylpropionic acid, and 1.25 g (6.5 mmol) of dicyclohexylcarbodiimide in 10 ml of pyridine was stirred at room temperature for 3 hr. Water (3 ml) was added and the mixture was stirred for 5 hr. Dicyclohexylurea was filtered off and the filtrate was evaporated to a brown gum, which was freed of pyridine by several evaporations of ethanol, and then taken up in ethyl acetate and chromatographed on silica gel (2  $\times$  43 cm) with ethyl acetate. Fractions containing material with *R*<sub>f</sub> 0.63 (tlc, EtOAc) were pooled and concentrated, and the residue was rechromatographed. On precipitation of the resulting product from ethyl acetate by addition of hexane, 0.493 g (50%) of the title compound was obtained; mp 112–116°; infrared bands at 2.93, 5.93, 8.60, 13.34, and 14.28  $\mu$ .

*Anal.* Calcd for C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O<sub>7</sub>: C, 72.66; H, 5.63; N, 4.34. Found: C, 72.57; H, 5.74; N, 4.43.

**3'-O-( $\beta$ -Benzoylpropionyl)thymidine (3)** was obtained by heating 0.493 g of 3'-O-( $\beta$ -benzoylpropionyl)-5'-O-tritylthymidine in 10 ml of 80% aqueous acetic acid on a steam bath for 20 min, evaporating the solvent at reduced pressure, chromatographing the residue on silica gel (2  $\times$  45 cm) with ethyl acetate and 50% methanol-ethyl

acetate, and recrystallizing from ethyl acetate the product from fractions showing *R*<sub>f</sub> 0.40 (tlc, EtOAc); weight of 3, 0.200 g (66%); mp 161–162°; infrared bands at 5.94, 8.17, 8.55, and 9.03  $\mu$ .

*Anal.* Calcd for C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>O<sub>7</sub>: C, 59.70; H, 5.51; N, 6.96. Found: C, 60.18; H, 5.80; N, 7.29.

In a large-scale preparation, 41 g (80 mmol) of 5'-O-(*p*-methoxytrityl)thymidine<sup>9</sup> was converted in a similar fashion to 3'-O-( $\beta$ -benzoylpropionyl)-5'-O-(*p*-monomethoxytrityl)thymidine, which without purification by chromatography was hydrolyzed directly to 3'-O-( $\beta$ -benzoylpropionyl)thymidine. After chromatography of the product on silica gel and recrystallization from ethyl acetate (500 ml)-tetrahydrofuran (20 ml), the 3'-O-( $\beta$ -benzoylpropionyl)thymidine weighed 18.1 g (56%); mp 158.5–160°.

**Reaction of Thymidine with Excess  $\beta$ -Benzoylpropionic Acid.** A solution of 0.484 g (2.0 mmol) of thymidine, 1.07 g (6.0 mmol) of  $\beta$ -benzoylpropionic acid, and 1.65 g (8.0 mmol) of dicyclohexylcarbodiimide in 15 ml of pyridine was stirred at room temperature. At the end of 5 hr and again after 8 hr of reaction, additional portions of  $\beta$ -benzoylpropionic acid (0.360 g, 2.0 mmol) and dicyclohexylcarbodiimide (0.412 g, 2.0 mmol) were added. The reaction was allowed to continue overnight and was then terminated by addition of 5 ml of water. The mixture was worked up as in the preparation of 3. Chromatography on silica gel (4  $\times$  47 cm) with ethyl acetate and mixtures of ethyl acetate and tetrahydrofuran (increasing percentages of THF) yielded two products. One was 3'-O,5'-O-bis( $\beta$ -benzoylpropionyl)thymidine (7): *R*<sub>f</sub> (tlc, EtOAc) 0.50; 282 mg (25%); mp 149–150°.

*Anal.* Calcd for C<sub>30</sub>H<sub>30</sub>N<sub>2</sub>O<sub>9</sub>: C, 64.05; H, 5.38; N, 4.98. Found: C, 64.48; H, 5.71; N, 5.56.

The other product was 5'-O-( $\beta$ -benzoylpropionyl)thymidine (6); mp (recrystallized from EtOAc) 158–159°; 0.108 g (14%).

*Anal.* Calcd for C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>O<sub>7</sub>: C, 59.70; H, 5.51; N, 6.96. Found: C, 60.19; H, 5.61; N, 7.13.

**Reaction of 7 with Hydrazine.** Diester 7 (0.250 g, 0.45 mmol) was stirred overnight with 26  $\mu$ l of 85% hydrazine hydrate (~0.45 mmol) in 0.30 ml of pyridine-acetic acid (4:1). After evaporation of the solvent the residue was chromatographed on silica gel with ether, ethyl acetate, and mixtures of ethyl acetate and tetrahydrofuran to give 49 mg of 7 (*R*<sub>f</sub> in EtOAc 0.54); 23 mg of the 3'-O ester, 3 (*R*<sub>f</sub> in EtOAc 0.38), 47 mg of the 5'-O ester, 6 (*R*<sub>f</sub> in EtOAc 0.28), and 25 mg of a mixture of 3 and 6.

**3'-O-(Benzoylformyl)-5'-O-tritylthymidine (4).** To 0.968 g (2.0 mmol) of 5'-O-tritylthymidine in 5 ml of pyridine was added 0.28 ml (2.1 mmol) of benzoylformyl chloride. Ethanol (0.5 ml) was added after the mixture had stood overnight, the solvent was evaporated, and the residue was chromatographed on silica gel with ethyl ether. Material with *R*<sub>f</sub> (ethyl ether) 0.40 was collected and precipitated from ethyl acetate by addition of hexane to give 1.06 g (86%) of 4; softens 98°, mp 100–103°.

*Anal.* Calcd for C<sub>27</sub>H<sub>32</sub>N<sub>2</sub>O<sub>7</sub>: C, 72.07; H, 5.34; N, 4.54. Found: C, 71.91; H, 5.32; N, 4.57.

**3'-O-Benzoylformylthymidine (5)** was obtained by heating 1.04 g of 4 in 8 ml of acetic acid and 2 ml of water for 20 min on a steam bath. It was isolated as in the case of 3; mp 77–80° with softening at 75°; weight 0.670 g (94%).

*Anal.* Calcd for C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub>: C, 57.75; H, 4.85; N, 7.48. Found: C, 57.29; H, 4.86; N, 7.36.

**5'-O-( $\beta$ -Benzoylpropionyl)thymidine (6) from 5.** A mixture of 0.534 g (1.42 mmol) of 3'-O-benzoylformylthymidine, 0.582 g (3.27 mmol) of  $\beta$ -benzoylpropionic acid, and 0.90 g (4.4 mmol) of dicyclohexylcarbodiimide in 6 ml of pyridine was stirred at room temperature overnight, mixed with water (0.5 ml), and, after 15 min of stirring, filtered. The filtrate was poured into 40 ml of water and extracted with chloroform (two 50-ml portions). On drying (Na<sub>2</sub>SO<sub>4</sub>) and concentration of the extract an oil was obtained, which on chromatography (silica gel with ether, ethyl acetate, and tetrahydrofuran) yielded an oil with *R*<sub>f</sub> (Et<sub>2</sub>O) 0.15 and *R*<sub>f</sub> (EtOAc) 0.53. Precipitation from ethyl acetate with hexane gave 0.348 g of the crude diester (mp ~68°). The benzoylformyl group was removed by stirring 0.300 g of this diester in 3 ml of pyridine and 1.5 ml of water overnight. Evaporation of the solvent and recrystallization of the residue from ethyl acetate gave 154 mg (31%) of 5'-O-( $\beta$ -benzoylpropionyl)thymidine: mp 155–156°. A mixture of this compound and the 3'-O isomer (3, mp 160–161°) melted in the range of 135–140° whereas the melting point of a mixture of this compound and 6 prepared by direct esterification of thymidine with  $\beta$ -benzoylpropionic acid showed no depression.

(6) H. Schaller and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3841 (1963).

(7) R. Shapiro and S. C. Agarwal, *ibid.*, **90**, 474 (1968).

(8) G. Weimann and H. G. Khorana, *ibid.*, **84**, 419 (1962).

(9) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *ibid.*, **85**, 3821 (1963).

**N<sup>6</sup>,3'-O,5'-O-Trisisobutyloxycarbonyldeoxycytidine (9).** Isobutyl chloroformate (0.35 ml, 2.6 mmol) was added dropwise with stirring to 150 mg (0.66 mmol) of 2'-deoxycytidine in 5 ml of anhydrous pyridine. After 4 hr the solvent was evaporated at reduced pressure at room temperature, and the solid residue, after several additions and evaporations of ethanol, was taken up in a minimum volume of chloroform and applied to a silica gel column (2 × 45 cm). Elution with ethyl acetate yielded (50–150-ml fraction) a component with  $R_f$  0.5 (EtOAc). This product (9) was precipitated by addition of hexane and chilling; weight 248 mg (72%); mp 119–120°; infrared bands at 2.71, 6.01, 6.40, 6.67, 7.9 (broad), and 8.24 (broad)  $\mu$ .

*Anal.* Calcd for C<sub>34</sub>H<sub>37</sub>N<sub>3</sub>O<sub>10</sub>: C, 54.64; H, 7.07; N, 7.96. Found: C, 54.82; H, 7.13; N, 7.98.

**N<sup>6</sup>-Isobutyloxycarbonyldeoxycytidine (10)** was obtained by stirring 2.5 ml of 0.8 *M* aqueous sodium hydroxide with 90 mg (0.17 mmol) of 9 in 2.5 ml of dioxane for 30 min at room temperature. The solution was then neutralized with pyridinium Dowex 50-X resin and, after filtration, was concentrated *in vacuo*. On addition of hexane and standing in a refrigerator, the resulting gum solidified; weight 34 mg (61%); mp 117–119°; infrared bands at 3.0, 5.71, 6.05, 6.45, 6.65, 8.2 (broad)  $\mu$ .

*Anal.* Calcd for C<sub>14</sub>H<sub>21</sub>O<sub>6</sub>: C, 51.40; H, 6.43; N, 12.85. Found: C, 51.73; H, 6.30; N, 13.37.

That the isobutyloxycarbonyl group can be removed satisfactorily from the N<sup>6</sup> position was shown by a reaction of 0.5 mmol of 10 with 7.5 ml of ammonium hydroxide and 2.5 ml of pyridine for 48 hr. Chromatography on aliquots showed that 10 had reacted completely to give a product with the same  $R_f$  as deoxycytidine ( $R_f$  0.69 for paper chromatography with isopropyl alcohol–ammonium hydroxide–water 7:1:2 and  $R_f$  0.21 with *n*-butyl alcohol–water 86:14). The major portion of the reaction mixture was evaporated–washed with chloroform, and taken up in 20 ml of water. On lyophilization 89 mg (78%) of deoxycytidine was obtained as a white powder. The hydrochloride salt prepared from this substance melted at 167–172° dec (not depressed when the sample was mixed with authentic deoxycytidine hydrochloride) and gave an infrared spectrum identical with that of deoxycytidine hydrochloride.

**N<sup>6</sup>-Isobutyloxycarbonyl-3'-O-( $\beta$ -benzoylpropionyl)-5'-O-(*p*-methoxytrityl)deoxycytidine (11).** To a solution of 527 mg (2 mmol) of deoxycytidine hydrochloride and 0.28 ml (2 mmol) of triethylamine in 15 ml of pyridine was added dropwise 1.1 ml (~8 mmol) of isobutyl chloroformate. The mixture was worked up and converted to N<sup>6</sup>-isobutyloxycarbonyldeoxycytidine as described in the preceding section. The crude gum obtained was mixed directly with 740 mg (2.2 mmol) of *p*-monomethoxytrityl chloride in 6 ml of pyridine. After 2 hr, additional *p*-methoxytrityl chloride (154 mg, 0.5 mmol) was added and the mixture was stirred for 11 hr at room temperature. Ethanol was added and stirring continued; then the

solvent was evaporated and the residue, after evaporation several times from ethanol, was taken up in the minimum volume of chloroform and chromatographed on silica gel with ethyl acetate–tetrahydrofuran as eluent (ranging from pure ethyl acetate to pure tetrahydrofuran). The fractions containing only material with  $R_f$  0.32 (EtOAc) were pooled and concentrated to yield the 5'-O-*p*-methoxytrityl derivative; weight 670 mg; mp 99–103°. A portion of this substance (300 mg, 0.50 mmol) was stirred for 22 hr at room temperature with 267 mg (1.5 mmol) of  $\beta$ -benzoylpropionic acid and 2.0 mmol of dicyclohexylcarbodiimide in 3 ml of pyridine. Water (0.5 ml) was added, and the mixture was stirred for 1 hr. Dicyclohexylurea was removed by filtration, and the filtrate was concentrated to a gum which was purified by chromatography on silica gel (2.5 × 45 cm) with solvents ranging from ether through ether–ethyl acetate mixtures to ethyl acetate and through mixtures of ethyl acetate–tetrahydrofuran to tetrahydrofuran. Fractions containing only the product with  $R_f$  0.53 (EtOAc) were pooled and concentrated to yield a gum which was crystallized from benzene–hexane. The yield of 11 was 175 mg (26%); mp 79°; infrared bands at 5.71, 5.95, 6.12, 6.40, 6.63, and 8.23  $\mu$ .

*Anal.* Calcd for C<sub>44</sub>H<sub>48</sub>N<sub>3</sub>O<sub>9</sub>: C, 68.70; H, 5.94; N, 5.54. Found: C, 68.82; H, 6.03; N, 5.59.

**N<sup>6</sup>-Isobutyloxycarbonyl-3'-O-( $\beta$ -benzoylpropionyl)deoxycytidine (12)** was obtained by stirring 100 mg of 11 with 1.6 ml of acetic acid and 0.4 ml of water for 4 hr at room temperature. On evaporation of the solvent and chromatography of the residue an oil was obtained which on standing in 4 ml of ethyl acetate in the refrigerator yielded 37 mg (57%) of 12; mp 160–161°; infrared bands at 5.72, 5.92, and 6.03  $\mu$ .

*Anal.* Calcd for C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>8</sub>: C, 59.20; H, 5.95; N, 8.62. Found: C, 58.87; H, 6.00; N, 8.70.

As a test of the cleanness of the unblocking step, 24 mg of 12 was stirred with 0.74 ml of ammonium hydroxide and 0.25 ml of pyridine for 48 hr. The solvent was evaporated and the residue subjected to paper chromatography with solvent F. Only two spots were observable in ultraviolet light. One corresponded to deoxycytidine ( $R_f$  0.71) and the other to  $\beta$ -benzoylpropionamide ( $R_f$  0.89). The material in the spot at  $R_f$  0.71 was eluted with water and heated at 65° for 18 hr with 178 mg of ninhydrin<sup>7</sup> in water (total volume 3 ml) in order to establish that the amine group was in fact present in the compound with  $R_f$  0.71. The solvent was evaporated and the residue was dissolved in acetone. On addition of ether a precipitate formed. It was collected by centrifugation, dissolved in ethanol, and subjected to thin layer chromatography on a cellulose slide with *n*-butyl alcohol–water (86:14). In addition to a spot at 0.91 corresponding to ninhydrin, a spot at 0.48 for the product of condensation of ninhydrin with deoxycytidine was observed. No other spots were found. This test showed that ammonium hydroxide indeed converts 12 to deoxycytidine.